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ORIGINAL ARTICLE

Changes in gas exchange and antioxidant metabolism on rice leaves infected by *Monographella albescens*

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Abstract The present study aimed to do an in-depth analysis of the effect of leaf scald on rice physiology by combining gas exchange measurements, photosynthetic pigment pools and examining the activities of enzymes involved in the antioxidant system. The size of leaf scald lesions increased from 3.1 mm at 24 h after inoculation (hai) to 25.2 mm at 120 hai. For the inoculated plants, net CO_2 assimilation rate, stomatal conductance to water vapor and transpiration rate decreased while internal CO_2 concentration increased in comparison to the not inoculated ones. The concentrations of chlorophyll *a*, chlorophyll *b*, total chlorophyll (*a* + *b*) and carotenoids decreased while the concentrations of malondialdehyde and hydrogen peroxide increased in the inoculated plants. The superoxide dismutase, ascorbate peroxidase, catalase, peroxidase and glutathione reductase activities significantly increased for the inoculated plants in comparison to non-inoculated ones. The results of this study show that infection by *M. albencens* negatively impacted the photosynthesis of rice leaves. Indeed, the CO_2 fixation capacity was dramatically reduced during fungal infection, which was deeply associated with lower concentrations of photosynthetic pigments in a scenario where a more efficient antioxidative system was detrimental to remove reactive oxygen species generated over the course of fungal infection.

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Introduction

Leaf scald, caused by the fungus *Monographella albescens* (Samuels and I. C. Hallet) = *Rhynchosporium oryzae* (Hashiola and Yokogi), is one of the major diseases affecting rice production worldwide (Ou 1985). The symptoms of leaf scald are zonate or oblong colored olive lesions with light brown halos without well-defined margins from the leaf tips or edges (Filippi et al. 2005). Upon lesion coalescence, large parts of the leaf blade become blight and dry out very quickly giving the leaf a scalded appearance, which negatively impacts photosynthesis (Nunes et al. 2004; Filippi et al. 2005). Periods of intense rain and prolonged foliar dew, temperatures ranging from 24 to 28 °C, close spacing and excess nitrogen are the most favorable conditions for the occurrence of leaf scald epidemics. Leaf scald has been managed by avoiding the excess of nitrogenous fertilizer, using resistant cultivars, non-infected seeds and their treatment with fungicides as well as foliar sprays of fungicides (IRRI 1983; Ou 1985; Groth 1992).

Photosynthesis is the major physiological process on plants affected by foliar pathogens (Bastiaans 1991; Bassanezi et al. 2002; Resende et al. 2012; Debona et al. 2014). Therefore, the proper assessment of the photosynthetic performance of plants under pathogen infection by examining the leaf gas exchange parameters net CO_2 assimilation rate (A), stomatal conductance to water vapor (g_s), transpiration rate (E) and internal CO_2 concentration (C_i) can provide insights into the mechanisms underlying their interaction (Rolfe and Scholes 2010).

Considering that plants are constantly challenged by several types of abiotic and biotic stresses, they efficiently need to modulate their antioxidative system to avoid any detrimental effect on both growth and yield. Plants respond against pathogen infection by activating defense mechanisms that redirect the derivatives of the primary carbon metabolism in favor of secondary metabolism (Bolton 2009). Pathogen infection causes a decrease in the photosynthetic rate usually associated with damage to the photosynthetic apparatus and increased excitation energy that exceeds the amount needed for photosynthetic metabolism, which ultimately causes the reduction of molecular oxygen and leads to the formation of reactive oxygen species (ROS) (Bassanezi et al. 2002; Kumudini et al. 2008; Behr et al. 2010; Iqbal et al. 2012). The superoxide ($O^{\bullet}{}_2^-$), hydroxyl ($OH^{\bullet}$), hydrogen peroxide (H_2O_2) and singlet oxygen ($_1O^2$) radicals are the most important ROS generated during fungal pathogenesis, which are capable of causing lipid peroxidation and protein denaturation (Asada 1999; Yu and Rengel 1999; Lima et al. 2002) besides causing damage to photosynthetic pigments and nucleic acids (Moller 2001).

To protect the photosynthetic apparatus against oxidative stress, plants have developed an antioxidant system that functions by increasing the concentrations of xanthophylls, carotenoids and other small molecules such as ascorbic acid and glutathione, as well as increasing the activities of several antioxidant enzymes (Asada 1999). In higher plants, the most important enzymes involved in the antioxidant system are superoxide dismutase (SOD), which catalyzes the dismutation of O_2^- and maintains its low levels due to the production of O_2 and H_2O_2; catalase (CAT), which converts H_2O_2 to H_2O and O_2; and enzymes of the glutathione-ascorbate cycle such as ascorbate peroxidase (APX), which catalyzes the oxidation of ascorbate to monodehydroascorbate (MDHA) using H_2O_2 as oxidant (Jimenez et al. 2002); ascorbate is oxidized and then reduced by glutathione, which is produced from oxidized glutathione catalyzed by glutathione reductase (GR) with the consumption of nicotinamide adenine dinucleotide phosphate (NADPH) (Apel and Hirt 2004). Finally, peroxidases (POX) performs the catalysis and biosynthesis of lignin generating H_2O_2 from nicotinamide adenine dinucleotide (NADH) (Goldberg et al. 1985) and also by the oxidation of phenolics (Fry 1986). The production of ROS is an important plant defense strategy against pathogen infection (Magbanua et al. 2007), but their accumulation caused by an imbalance between production and removal can damage the host tissue (Lima et al. 2002; Scandalios 2011).

Considering the negative impact of leaf scald on rice production and that more basic information regarding the rice-*M. albencens* interaction is needed, the present study aimed to do an in-depth analysis of the effect of leaf scald on rice physiology by combining gas exchange measurements, photosynthetic pigment pools and examining the activities of enzymes involved in the antioxidant system.

Materials and methods

Plant growth

Rice seeds from the Primavera cultivar, which is susceptible to leaf scald (Tatagiba et al. 2014), were surface-sterilized in 10 % (v/v) NaOCl for 2 min, rinsed in sterilized water for 3 min and germinated on distilled water-soaked germtest paper in a germination chamber (MA-835/2106UR, Marconi) at 25 °C for 6 days. Three germinated seedlings were transplanted in plastic pots containing 3 kg of substrate composed of an 1:1:1 mixture of pine bark, peat and expanded vermiculite (Tropstrato, Vida Verde). Plants were grown in a greenhouse (temperature 28 ± 2 °C during the day and 23 ± 2 °C at night, relative humidity 73 ± 5 %). A total of 1.63 g of calcium phosphate monobasic was added to each plastic pot. Plants were fertilized weekly with 50 mL of a nutrient solution containing, in mg/L, 192 KCl; 104.42 K_2SO_4; 150.35 $MgSO_4.7H_2O$; 61 urea; 100 NH_4NO_3; 0.27 $NH_4MO_7.24.4$ H_2O; 1.61 H_3BO_3; 6.67 $ZnSO_4.7H_2O$; 1.74 $CuSO_4.5H_2O$; 4.10 $MnCl_2.4H_2O$; 4.08 $FeSO_4.7H_2O$ and 5 disodium EDTA. The nutrient solution was prepared using deionized water. Plants were watered as needed.

Inoculation of the plants with *M. albescens*

An isolate of *M. albescens* (UFV/DFP-*Ma* 022), obtained from the symptomatic leaves of rice plants of the Bonança cultivar, was used to inoculate the plants (Tatagiba et al. 2014). This isolate was preserved in glass vials containing potato-dextrose-agar (PDA), covered with mineral oil and maintained at 4 °C. Plugs of PDA with fungal mycelia were transferred to Petri dishes containing PDA and maintained in a growth chamber (MA-835/2106UR) at 25 °C with a 12 h photoperiod for 15 days. Five plants per replication of each treatment were inoculated with *M. albescens* after growing for 45 days (Matsuo and Hoshikama 1993) in plastic pot filled with 2 kg of a substrate made from a 1:1:1 mixture of pine bark, peat, and expanded vermiculite (Tropstrato). Three PDA discs (0.3 cm^2) containing *M. albescens* mycelia were placed equidistantly on the adaxial side of the 7th, 8th and 9th leaves (from the base to the apex) of each plant and gently pressed onto the surface. Immediately after inoculation, the plants were transferred to a plastic mist growth chamber (MGC) inside a greenhouse The temperature inside of the MGC ranged from 25 ± 4 °C (day) to 21 ± 2 °C (night) and the relative humidity was maintained at 92 ± 3 % using a misting system (model NEB-100, KGF Co.) that sprayed mist above the plant canopies every 30 min. The relative humidity and temperature were measured with a thermo-hygrograph (TH-508, Impac). The maximum natural photon flux density at the plant canopy height was approximately 950 μmol/m^2/s. Non-

inoculated plants were kept in a separate MGC, but were exposed to the same conditions as the inoculated plants.

Evaluation of expanding lesions

The expansion (in mm) of the three leaf scald lesions on the adaxial surfaces of the 7th, 8th and 9th leaves, from the base to the apex, was measured using an electronic digital caliper (China Suppliers) at 24, 48, 72, 96 and 120 h after inoculation (hai). The values from each replication consisted of the arithmetic mean of the values for the three lesions on each leaf.

Leaf gas exchange evaluations

Leaf gas exchange parameters were determined using a portable open-flow gas exchange system (LI-6400XT, LI-COR). Net CO_2 assimilation rate (A), stomatal conductance to water vapor (g_s), transpiration rate (E) and internal CO_2 concentration (C_i) were measured on attached leaves (7th, 8th and 9th leaves from the base to the apex per replication for each treatment) at 24, 72 and 120 hai. The values from each replication consisted of the arithmetic mean of evaluations (three lesions on each one of the three evaluated leaves). The measurements were performed from 09:00 to 10:30 h (solar time), a time when A was at its maximum, under artificial photosynthetically active radiation, i.e., 1,000 μmol photons/m^2/s at the leaf level and 400 μmol CO_2/mol air. The non-inoculated plants were also evaluated at these times. All of the measurements were performed at 25 °C and the vapor pressure deficit was maintained at approximately 1.0 kPa; the amount of blue light was set to 10 % of the photosynthetic photon flux density to optimize the stomatal aperture.

Biochemical assays

Leaf tissue (≈4 cm^2) from the 7th, 8th and 9th leaves, counted from the base to the top, of plants from each treatment and replication were collected at 24, 48, 72, 96 and 120 hai to determine the enzymes activities and the concentrations of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) and only at 120 hai to determined the concentration of pigments. Leaf tissue was also collected from non-inoculated plants at these sampling times. Leaf samples were kept in liquid nitrogen during sampling and then stored at −80 °C until further analysis.

Determination of pigment concentration on leaf tissue

A total of 100 mg of leaf tissue was ground into a fine powder using a mortar and pestle with liquid nitrogen and the addition of 1 mg of calcium carbonate. Next, the fine powder was homogenized with 2 mL of aqueous acetone (80 %, v/v) for 1 min in a room with reduced light intensity. The suspension was filtered through Whatman #1 filter paper and the residue was washed four times with 80 % acetone. The volume was increased to 25 mL with the same solvent in a volumetric flask. The absorbance of the samples was recorded at 470, 646.8 and 663.2 nm and the concentrations of photosynthetic pigments (Chl_a, Chl_b and carotenoids) were estimated according to Lichtenthaler (1987).

Determination of enzymes activities

To determine the activities of superoxide dismutase (SOD, EC 1.15.1.1), ascorbate peroxidase (APX, EC 1.11.1.11), catalase (CAT, EC 1.11.1.6) and peroxidase (POX, EC 1.11.1.7), a total of 300 mg of leaf tissue (mix of the 12 leaves collected per replication of each treatment) was ground into a fine powder in a mortar and pestle with liquid nitrogen. The fine powder was homogenized in an ice bath in 2 mL of a solution containing 50 mM potassium phosphate buffer (pH 6.8), 0.1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM phenylmethylsulfonyl fluoride (PMSF) and 2 % (w/v) polyvinylpolypyrrolidone at 4 °C and the supernatant was used as a crude enzyme extract. To determine glutathione reductase (GR, EC 1.8.1.7) activity, a total of 300 mg of leaf tissue was ground as described above. The fine powder was homogenized in an ice bath in 2 mL of a solution containing 100 mM potassium phosphate buffer (pH 7.5), 0.1 mM EDTA, 1 mM *DL*-dithiothreitol, 1 mM PMSF and 2 % (w/v) PVPP. The homogenate was centrifuged as described previously.

SOD activity was determined by measuring its ability to photochemically reduce *p*-nitrotetrazole blue (NTB) (Del Longo et al. 1993). The reaction was started after the addition of 60 μL of the crude enzyme extract to 1.94 mL of a mixture containing 50 mM potassium phosphate buffer (pH 7.8), 13 mM methionine, 75 μM NTB, 0.1 mM EDTA and 2 μM riboflavin. The reaction occurred at 25 °C under a 15 W lamp light. After 10 min of light exposure, it was interrupted and the production of formazan blue, which resulted from the photoreduction of NTB, was monitored by the increase in absorbance at 560 nm in spectrophotometer (Evolution 60, Thermo Fisher Scientific) (Giannopolitis and Ries 1977). The reaction mixture for the control samples was kept in darkness for 10 min and the absorbance was measured at 560 nm. The values obtained were subtracted from the values obtained from the samples of the replications of each treatment exposed to light. One unit of SOD was defined as the amount of enzyme necessary to inhibit NBT photoreduction by 50 % (Beauchamp and Fridovich 1971).

APX activity was determined according to method of Nakano and Asada (1981). The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 6.8), 1 mM H_2O_2 and 0.8 mM ascorbate in a volume of 1.95 mL. The reaction was started after the addition of 50 μL of the crude

enzyme extract. The APX activity was measured by the rate of ascorbate oxidation at 290 nm for 1 min at 25 °C. An extinction coefficient of 2.8 mM^{-1} cm^{-1} (Nakano and Asada 1981) was used to calculate APX activity, which was expressed as μmol/min/mg of protein.

CAT activity was determined following the method of Cakmak and Marschner (1992). The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 6.8) and 20 mM H_2O_2 in a volume of 1.95 mL. The reaction was initiated after the addition of 50 μL of the crude enzyme extract and the CAT activity was determined by the rate of H_2O_2 decomposition at 240 nm for 1 min at 25 °C. An extinction coefficient of 36 M^{-1} cm^{-1} (Anderson et al. 1995) was used to calculate CAT activity, which was expressed as μmol/min/mg of protein.

POX activity was assayed following the colorimetric determination of pyrogallol oxidation according to Kar and Mishra (1976). The reaction mixture contained 25 mM potassium phosphate (pH 6.8), 20 mM pyrogallol and 20 mM H_2O_2 in a volume of 1.98 mL. The reaction was started after the addition of 15 μL of the crude enzyme extract and POX activity was determined through the absorbance of colored purpurogallin recorded at 420 nm for 1 min at 25 °C. An extinction coefficient of 2.47 mM^{-1} cm^{-1} (Chance and Maehley 1955) was used to calculate the POX activity, which was expressed as μmol of purpurogallin produced per min/mg of protein.

To determine GR activity, the reaction was started after the addition of 100 μL of the crude enzyme extract to a volume of 1.9 mL of a mixture containing 100 mM potassium phosphate (pH 7.5), 1 mM EDTA, 1 mM oxidized glutathione (GSSG) and 0.1 mM NADPH prepared in 0.5 mM Tris–HCl buffer (pH 7.5) according to Carlberg and Mannervik (1985). The decrease in absorbance was determined at 340 nm for 1 min at 30 °C. An extinction coefficient of 6.22 mM^{-1} cm^{-1} (Kar and Mishra 1976) was used to calculate the GR activity, which was expressed as μmol/min/mg of protein.

Determination of MDA

Oxidative damage in the leaf cells was estimated as the concentration of total 2-thiobarbituric acid (TBA) reactive substances and expressed as equivalents of malondialdehyde (MDA) according to Cakmak and Horst (1991). A total of 100 mg of leaf tissue was ground into a fine powder using a mortar and pestle with liquid nitrogen. The fine powder was homogenized in 2 mL of 0.1 % (w/v) trichloracetic acid (TCA) solution in an ice bath. The homogenate was centrifuged at 12.000 *g* for 15 min at 4 °C. After centrifugation, 0.5 mL of the supernatant was reacted with 1.5 mL of TBA solution (0.5 % in 20 % TCA) for 30 min in a boiling water bath at 95 °C. After this period, the reaction was stopped in an ice bath. The samples were centrifuged at 9,000 *g* for 10 min and the specific absorbance was determined at 532 nm. The nonspecific absorbance was estimated at 600 nm and subtracted from the specific absorbance value. An extinction coefficient of 155 mM^{-1} cm^{-1} (Heath and Packer 1968) was used to calculate the MDA concentration, which was expressed as nmol/g of fresh matter (FM).

Determination of the concentration of H_2O_2

A total of 100 mg of leaf tissue was macerated in liquid nitrogen in a mortar to obtain a fine powder and the obtained powder was homogenized in 2 mL of potassium phosphate buffer 50 mM (pH 6.5) and 1 mM hydroxylamine. The homogenate was centrifuged at 10,000 *g* for 15 min at 4 °C (Kuo and Kao 2003). A total of 100 mL of the supernatant was added to a reaction mixture consisting of $FeNH_4$ (SO_4) 100 mM, 25 mM sulfuric acid, xylenol orange 250 mM and sorbitol 100 mM in a final volume of 2 mL (Gerbicki and Gay 2000). Samples were kept in the dark for 30 min and the absorbance was measured at 560 nm. The controls for the reagents and crude extracts were prepared under the same conditions and subtracted from the sample. The H_2O_2 concentration was estimated based on a standard curve for H_2O_2 (Sigma-Aldrich).

Experimental design and statistical analysis

Two experiments were conducted in a completely randomized design with two treatments (non-inoculated plants and plants inoculated with *M. albencens*) and six replications. Each experimental unit consisted of one pot containing three plants. Data from each variable evaluated represented the mean value from two independent experiments. Data were subjected to an analysis of variance and the treatment means were then compared by the *t*-test ($P \leq 0.05$) using SAS software (Release 8.02 Level 02 M0 for Windows, SAS Institute, Inc.).

Results

Lesion expansion

The size of the leaf scald lesions increased from 3.1 mm at 24 hai to 25.2 mm at 120 hai.

Leaf gas exchange parameters

For the inoculated plants, *A* was significantly reduced by 73 and 80 %, respectively, at 72 and 120 hai in comparison to non-inoculated ones (Fig. 1a). Significant reductions of 71 and 77 %, respectively, at 72 and 120 hai occurred for g_s on inoculated plants in comparison to non-inoculated ones (Fig. 1b). Significant reductions of 55 and 77 % at 72 and

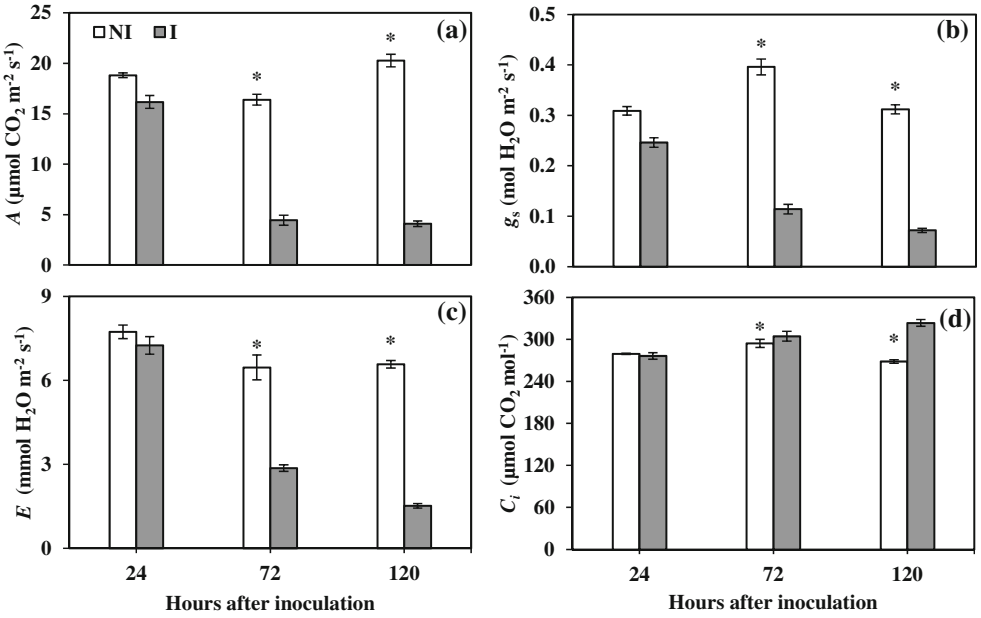


Fig. 1 Net CO_2 assimilation rate (A) (**a**), stomatal conductance to water vapor (g_s) (**b**), transpiration rate (E) (**c**) and internal CO_2 concentration (C_i) (**d**) determined in the leaves of rice plants non-inoculated (NI) and inoculated (I) with *Monographella albescens*. Means of the NI and I treatments followed by an asterisk (*) are significantly different by *t*-test at 5 % probability. The error bars represent standard deviation of the means

120 hai, respectively, for E occurred for the inoculated plants in comparison to non-inoculated ones (Fig. 1c). Increases of 3 and 17 % for C_i at 72 and 120 hai, respectively, occurred for the inoculated plants in comparison to non-inoculated ones (Fig. 1d).

Concentration of pigments

Significant reductions of 63, 59, 62 and 24 %, respectively, for the concentrations of Chl_a, Chl_b, total chlorophyll and carotenoids occurred at 120 hai for the inoculated plants in comparison to non-inoculated ones (Fig. 2).

Activities of antioxidant enzymes

For APX activity, significant increases of 49, 32, 23 and 14 %, respectively, at 48, 72, 96 and 120 hai occurred for the inoculated plants in comparison to non-inoculated ones (Fig. 3a). POX activity significantly increased by 83, 84, 67 and 79 %, respectively, at 48, 72, 96 and 120 hai for the inoculated plants in comparison to non-inoculated ones (Fig. 3b). CAT activity was significantly higher for inoculated plants compared to non-inoculated plants at 48 and 72 hai, but significantly lower for the inoculated plants compared to the non-inoculated ones at 120 hai (Fig. 3c). There were significant increases in SOD activity of 21 and 19 % at 48 and 120 hai, respectively, for the inoculated plants in comparison to non-inoculated ones (Fig. 3d). GR activity increased significantly by 61, 82, 80 and 41 %, respectively, at 48, 72, 96 and 120 hai for the inoculated plants in comparison to non-inoculated ones (Fig. 3e).

MDA and H_2O_2 concentrations

MDA concentration significantly increased by 43, 57 and 57 %, respectively, at 72, 96 and 120 hai for the inoculated plants in comparison to non-inoculated ones (Fig. 4a). There were significant increases of 59, 62, 45, 52 and 38 % on H_2O_2 concentration, respectively, at 24, 48, 72, 96 and 120 hai for the inoculated plants in comparison to non-inoculated ones (Fig. 4b).

Discussion

The present study confirms the negative effects of leaf scald on rice physiology by examining leaf gas exchange

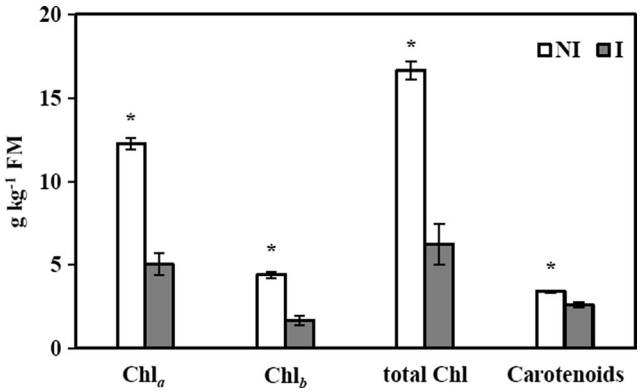
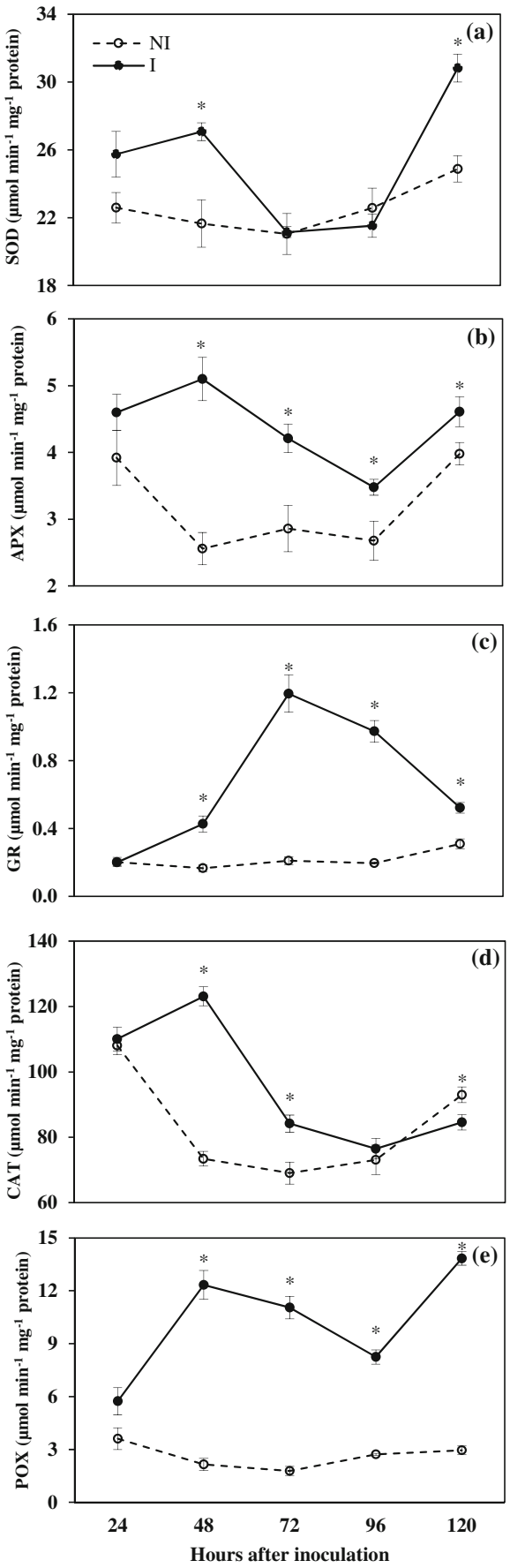


Fig. 2 Concentrations of chlorophyll *a* (Chl_a), chlorophyll *b* (Chl_b), total chlorophyll (Chl_{a+b}) and carotenoids in the leaves of rice plants non-inoculated (NI) and inoculated (I) with *Monographella albescens* at 120 h after inoculation. Means of the NI and I treatments followed by an asterisk (*) are significantly different by *t*-test at 5 % probability. The error bars represent standard deviation of the means. *FM* fresh matter



◀ **Fig. 3** Activities of superoxide dismutases (SOD) (**a**), ascorbate peroxidase (APX) (**b**), glutathione reductase (GR) (**c**), catalase (CAT) (**d**) and peroxidase (POX) in the leaves of rice plants non-inoculated (NI) and inoculated (I) with *Monographella albescens*. Means of the NI and I treatments followed by an asterisk (*) are significantly different by *t*-test at 5 % probability. The error bars represent standard deviation of the means

parameters and the concentration of photosynthetic pigments (Tatagiba et al. 2015) and brings novel information on how the antioxidative metabolism on rice leaf blades is altered during the infection process of *M. albencens*. The values in A, g_s and E were lower in leaves infected by *M. albencens*, indicating the adverse effect of fungal infection on these gas exchange parameters, especially A. The limitation on photosynthesis on infected leaves may be related to reduced stomatal aperture and lower concentrations of photosynthetic pigments. Increases in C_i on leaves of plants infected with *M. albencens* may be associated with a decrease on the amount of green leaf tissue due to the intense necrosis and chlorosis. The possible closure of stomata on leaves infected by *M. albencens* may have contributed to reduce E. The reduction of photosynthesis in leaves infected by pathogens can occur due to changes in opening and closure of stomata, hindering CO_2 diffusion in the mesophyll and reduction or destruction of chlorophylls or chloroplasts, which results in chlorosis and necrosis of the leaf tissue (Rolfe and Scholes 2010).

Considering that carbon fixation was possibly reduced on the leaves of plants infected by *M. albencens*, the energy surplus resulting from the excitationof light absorbed by chloroplasts during photosynthesis must be dissipated to avoid photo-oxidative damage in the leaves. Indeed, the photosynthetic reduction of O_2, which can occur through photorespiration and the Mehler-peroxidase pathway, may provide protection to the excess of light, acting in the dissipation of excess energy in the photosynthetic apparatus (Biehler and Fock 1996). It is plausible that the energy available for photosynthesis on infected leaves may be directed by the host for reduction of O_2 and, consequently, production of ROS. Indeed, the plant can drive its metabolism to produce ROS in a tentative to reduce further fungal growth.

The high SOD activity in the early and at advanced stages of fungal infection may have contributed to the protection against oxidative stress in infected leaves. As expected, an increase in the production of H_2O_2 was associated with an increase in SOD activity, which can be coupled to the rapid removal of H_2O_2 by other disposal systems, thus minimizing H_2O_2 cytotoxicity (Perl et al. 1993). Increases in SOD activity and high concentrations of ROS may be also a strategy used by pathogens to allow an efficient colonization of host tissues to obtain the desirable nutrients for their growtn (Ehsani-Moghaddam et al. 2006; Govrin and Levine 2000). Kuźniak and Sklodowska (2005) noted that in tomato leaves, SOD activity increased during the early stages of *B. cinerea*

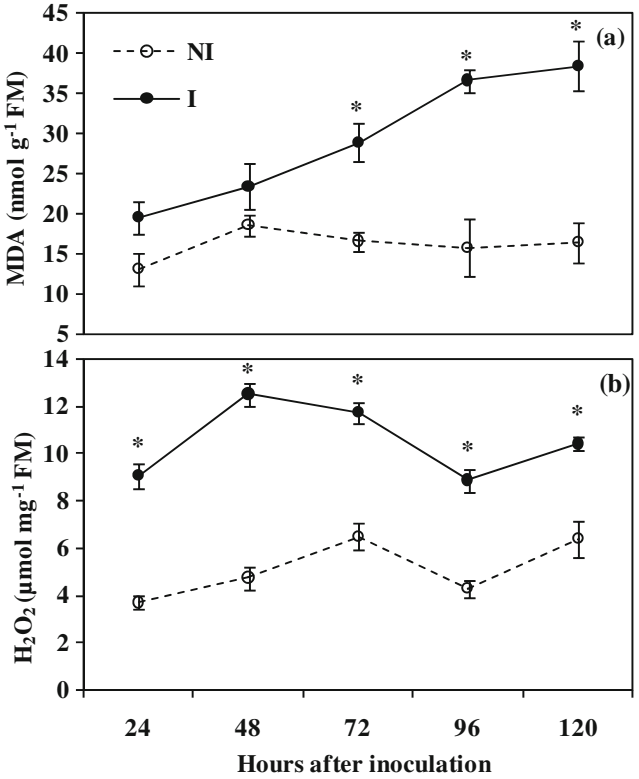


Fig. 4 Concentrations of malondialdehyde (MDA) (**a**) and hydrogen peroxide (H_2O_2) (**b**) in the leaves of rice plants non-inoculated (NI) and inoculated (I) with *Monographella albescens*. Means of the NI and I treatments followed by an asterisk (*) are significantly different by *t*-test at 5 % probability. The error bars represent standard deviation of the means. *FM* fresh matter

infection, but decreased as necrotic lesions expanded. By contrast, on wheat leaves infected with *Pyricularia oryzae*, SOD activity increased in comparison to non-infected leaves (Debona et al. 2012).

APX activity dramatically increased for plants infected with *M. albencens* in relation to non-inoculated plants. Increases in the levels of *apx* transcripts and on APX activity are well documented in the literature for many plant-pathogen interactions (El-Zahabi et al. 1995; Huckelhoven et al. 2001; Agrawal et al. 2002; Harrach et al. 2008; Mohaghegh et al. 2011; Resende et al. 2012). Although APX activity increased, H_2O_2 concentration was kept high from 24 to 120 hai on infected leaves contributing, therefore, to maximize the deleterious effects of the fungus to rice leaf physiology. Plants can use the ROS available on their tissue to increase their level of resistance against diseases by inhibiting pathogen growth and also strengthening the cell wall, which may reduce membrane fluidity (Pascholati et al. 2008). However, results from the present study indicate that *M. albencens* was favored by the increase in H_2O_2 concentration. Moreover, as a strategy to increase the colonization of rice leaf tissue, non-host selective toxins released by this necrotrophic fungus (Araújo et al. 2015) could reduce the amount of O_2, resulting in the accumulation of ROS and increasing the permeability of the host cell wall membranes, thus favoring fungal growth. In the meantime, APX and GR activities increase in an attempt to reduce the oxidative stress resulting from *M. albencens* infection. APX and GR are components of the ascorbate-glutathione cycle and with the participation of dehydroascorbate reductase (DHAR) and monodehydroascorbate reductase (MDHAR, facilitate the removal of excess H_2O_2 and other ROS) (Foyer et al. 1997; Mittler 2002). Although APX and GR play a detrimental role in the antioxidant system of plants, their relationship to host resistance against pathogens need to be further investigated. According to El-Zahabi et al. (1995), there was no change on GR activity in the leaves of plants from three barley cultivars infected with virulent and avirulent isolates of *Blumeria graminis* f. sp. *hordei*.

Among the enzymes involved in removing excess H_2O_2, CAT plays a key role (Mittler 2002). Increases in the concentration of H_2O_2 in *M. albencens* infected leaves may be associated with a reduction in CAT activity during fungal infection. In general, a reduction in CAT activity increases host resistance against pathogen attack by attempting to maintain the concentration of H_2O_2 at a high level (Magbanua et al. 2007). Thus, the role of CAT in a certain host-pathogen interaction appears to be more complex than in the case of an abiotic type of stress (Kuźniak and Sklodowska 2005). Vanacker et al. (1998) reported an increase in CAT activity in barley leaves infected with *Blumeria graminis* f. sp. *hordei*, while in tomato leaves CAT activity increased at the early stage of *Botrytis cinerea* infection and decreased as disease developed (Kuźniak and Sklodowska 2005).

High POX activity was important in an attempt to reduce the oxidative stress resulting from *M. albencens* infection. In addition to the involvement of POX in the removal of H_2O_2, this enzyme plays an important role in host defense, especially through host tissue lignification (Rauyaree et al. 2001). Debona et al. (2012) found that high POX activity was an important strategy of wheat plants to cope with *P. oryzae* infection.

Measuring the production of MDA, which is an indirect indicator of lipid peroxidation in the cell wall membrane, suggests that the high concentration of this metabolite in the leaves of rice plants over the course of *M. albescens* infection was the result of massive fungal colonization, which can also be indirectly confirmed by the greater values of lesion expansion. Indeed, the increase in H_2O_2 concentration, and possibly of other ROS during the infection process of *M. albencens*, may have contributed to the high MDA concentration. Thus, increasing the capacity of the antioxidant system of rice plants becomes important to eliminate ROS in leaf tissues infected by *M. albencens*.

In conclusion, the results of this study show that infection by *M. albencens* negatively impacted photosynthesis of rice

leaves. The CO_2 fixation capacity was reduced during fungal infection, which was clearly associated with lower concentrations of photosynthetic pigments in a scenario where a more efficient antioxidative system was detrimental to remove the ROS generated over the course of fungal infection.

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